Journal of Chemical Ecology, Vol. 28, No. 3, March 2002 (© 2002)

CHEMICAL DEFENSE IN THE PLANT BUG *Lopidea robiniae* (UHLER)

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(Received June 28, 2000; accepted October 31, 2001)

Abstract—Secretions from the metathoracic glands (MTG) of the black locust bug, *Lopidea robiniae* (Uhler) (Heteroptera: Miridae) contained six major compounds, including (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (*E*)-2-octenal, (*E*)-2-octen-1-ol (*E*)-2-heptenal, and (*Z*)-3-octen-1-ol. Males and females did not differ significantly in the relative compositions of identified compounds. In feeding trials, six bird species [robin (*Turdus migratorious*), blue jay (*Cyanocitta cristata*), brown thrasher (*Toxostoma rufum*), killdeer (*Charadrius vociferus*), starling (*Sturnus vulgaris*), and house wren (*Troglodytes aedon*)] demonstrated feeding aversions towards *L. robiniae*, implying that black locust bugs are chemically defended. Bugs discharged the liquid contents of their MTG when attacked, thereby producing a strong and distinct odor. Some birds immediately ejected bugs out of their mouth after biting them, suggesting that the MTG secretion was a deterrent.

Key Words—Aldehydes, alcohols, chemical defense, Heteroptera, Miridae, *Lopidea robiniae*, *Robinia pseudoacacia*, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (*E*)-2-octenal, (*E*)-2-octen-1-ol, (*E*)-2-heptenal, (*Z*)-3-octen-1-ol.

INTRODUCTION

The mirids are the largest family of Heteroptera, numbering over 1900 species in North America (McPherson, 1989) and 10,000 species worldwide (Schuh and Slater, 1995). Mirids predominately use crypsis and agile escape maneuvers, such

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as taking flight, dropping, and feigning death, to avoid predation (Aldrich, 1988). Although adults have metathoracic scent glands, (MTG) and the nymphs and some adults possess dorsal abdominal scent glands (DAG), the chemical ecology of only a few species has been examined (Aldrich, 1988; Millar et al., 1997; Millar and Rice, 1998; McBrien and Millar, 1999).

A common genus in the family Miridae is *Lopidea*, with more than 60 species occurring solely in the Western Hemisphere throughout much of North and Central America. *Lopidea* are different from other mirids in that most are relatively large (>5.0 mm) and are brightly colored with red, orange, or yellow on a black background, creating striking patterns (Asquith, 1993). For insects, large size and conspicuous coloration is often associated with chemical defense (Pasteels et al., 1983).

The black locust bug, *Lopidea robiniae* (Uhler), is common in the eastern United States and is found nearly everywhere its host tree, black locust, *Robinia pseudoacacia* L., grows. Adults are typically 6.0–6.5 mm in length and 2.1 mm in width. Males and females are aposematically colored orange–yellow with a broad black stripe that runs along the mid-dorsum from the tip of the head to the end of the abdomen (Knight, 1941). The nymphs are also aposematic with black wingpads on an orange–red body. Our observations suggest that, in Illinois, black locust bugs overwinter in the egg stage and hatch in early spring after their host tree produces new growth. In the fall, we have observed adults aggregating on goldenrod plants. In central Illinois, adults first appear in mid-June and become scarce by middle to late September. The adults emit a conspicuous odorous secretion when disturbed.

Interestingly, the black locust bug appears to be involved in a mimicry relationship with the adult stage of the locust leaf miner beetle *Odontota dorsalis* (Thurnburg) (Chrysomelidae). The larvae feed on black locust and the adult beetles closely resemble the black locust bug in color, pattern, and size. Additionally, both species occur at the same time of year and can be found together on the same plant where their ranges overlap.

In this paper, we identify the volatile chemical components and glandular source of the secretion in the black locust bug. Additionally, we examine the palatability of *L. robiniae* against various avian predators and provide evidence that the bugs are chemically defended and that the secretion functions as a feeding deterrent against predators.

METHODS AND MATERIALS

Insect Collection and Care

Adult black locust bugs, *Lopidea robiniae* (Uhler), were collected on black locust trees, *Robinia pseudoacacia* L., in and near Normal, Illinois, USA, from late July to mid-September 1998. Thereafter, the bugs were maintained until

needed in 2-liter translucent plastic jars at 25°C under a 14L:10D photoperiod and fed daily with fresh cuttings of black locust twigs and leaves, with the base of the twig inserted into a vial of water. Black locust bugs readily emit their secretions in response to even mild disturbances (see below). Therefore, during collecting, maintaining, and testing, the bugs were handled in an extremely gentle manner.

Chemical Analysis

Twenty *L. robiniae* adults were separated by sex using a dissecting microscope, then placed individually into glass GC autosampler vials (12 × 32 mm; Hewlett-Packard) and sealed with a Teflon-lined aluminum crimp cap. Before sealing the vials, the adults therein were agitated by gently poking them with a probe. Undisturbed male and female insects were also individually isolated in vials to serve as controls. We used solid-phase microextraction (SPME) (100 μm polydimethylsiloxane fiber; Supelco Inc., Bellefonte, Pennsylvania) to sample volatiles from individual vials. Sampling time was 30–45 min. Volatiles obtained from SPMEs of individual bugs were analyzed using a gas chromatograph (GC) (Hewlett-Packard model 5890 Series II) equipped with split/splitless injector run in splitless mode. The injector port was purged after 30 sec. Compounds were separated using a 30-m × 0.25-μm ID DB-5 column (J & W Scientific, Folsom, California) with a temperature program of 50°C for 1 min, followed by a ramp of 50°C to 250°C at 10°C/min, and remaining at 250°C for 6 min. Individual compounds were detected by flame ionization.

Mass spectra were obtained with a Hewlett-Packard 5973 MSD mass spectrometer. We introduced samples through a DB-SMS capillary column (30 m × 0.25 mm ID, 1.0-μm film) using a temperature program of 50°C for 1 min, followed by a ramp of 50°C to 250°C at 10°C/min, and remaining at 250°C for 6 min.

Authentic standards of (*E*)-2-hexenal (99%), (*Z*)-2-hexenal (97%), (*E*)-2-hexen-1-ol (96%), (*Z*)-2-hexen-1-ol (95%), (*E*)-3-hexen-1-ol (98%), (*Z*)-3-hexen-1-ol, (98%) (*E*)-2-heptenal (99%), (*Z*)-2-heptenal (97%), (*Z*)-3-octen-1-ol (94%), (*E*)-2-octenal (99%), (*Z*)-2-octenal (97%), (*E*)-2-octen-1-ol (96%), and (*Z*)-2-octen-1-ol (95%) were purchased from Aldrich Chemical Co., Milwaukee, Wisconsin. Relative concentrations of individual secretion components were determined from GC peak areas, after compensating for biases for alcohols and heavier compounds inherent in SPME sampling (Bartelt, 1997).

Behavioral Response toward Threats

We used a 3-mm-diam. wooden dowel to examine the behavioral response of *L. robiniae* to threats. Ten wild adults were individually tested in the field on a warm sunny day (33–35°C) by moving the tip of the dowel several times to within

0.5 cm of the insect, at a rate of 2 cm/sec, then catching the insects in a net, and finally, holding the insects between the fingers.

Predator Feeding Trials

We tested six bird species for their ability to feed on black locust bugs. Birds were obtained as nestlings and raised in the laboratory on a diet of various arthropods and cat food supplemented with bird vitamins, earthworms, fruit, snails, and small fish. The specific diet approximated the natural diet of each bird species. In all cases, birds were tested only after they had developed the ability to feed independently and discriminate among food items. All the birds had experienced a variety of both palatable and chemically defended prey in the week prior to testing. For the robin (*Turdus migratorious*), brown thrasher (*Toxostoma rufum*), and blue jay (*Cyanocitta cristata*), this included several encounters with black locust bugs; hence, these birds had already experienced *L. robiniae*. In contrast, the killdeer (*Charadrius vociferus*), starling (*Sturnus vulgaris*), and the house wrens (*Troglodytes aedon*) had never encountered black locust bugs when tested and hence were naive vis-à-vis this insect.

The birds were starved for 2–4 hr prior to testing, and each was tested independently in 0.3- to 1.0-m^3 cages. Generally, each bird was offered a series of live *L. robiniae* adults until the bird refused to attack further offerings. Then the bird was offered alternative live palatable arthropods (crickets, *Acheta domesticus* L., and/or mealworms, *Tenebrio molitor* L.) of approximately the same mass as the black locust bugs. In some trials, birds received bugs and palatable prey in alternation. In addition to these tests using solitary birds, we also introduced bugs into a 1-m^3 cage containing a group of six wrens. Each test was completed within 48 min. After the trials, birds were released in appropriate habitats.

Blue Jay, Brown Thrasher, and American Robin. During the trials, each bird was offered a series of live *L. robiniae* by placing individual bugs in a Petri dish in the cage until the bird rejected at least four in a succession. Then, each was offered a series of five mealworms and three crickets.

Killdeer. The killdeer was first given a control third-instar *A. domesticus* cricket to demonstrate hunger. Then, individual *L. robiniae* were offered successively until the bird no longer consumed the bugs. When this occurred, the bird was then offered another control cricket to verify that the bird was not simply satiated. If the control was eaten, another treatment was then offered. Uneaten bugs were removed 4 min after placing them in the cage with the bird. This protocol was continued until the bird repeatedly ate the control insects, but repeatedly rejected the bugs, at which time the experiment was ended.

European Starling. This bird was tested with a protocol identical to that of the killdeer (above) except that mealworms were used as controls. With this predator,

we also recorded time to attack (the time from introduction of each insect until the bird pecked at the insect).

House Wren. A Petri dish containing seven live black locust bugs was placed into the bird's cage. Ten minutes later (when the wren had stopped attacking the bugs), a second Petri dish was added that contained four black locust bugs, four small crickets, and one mealworm. Eleven minutes later a third Petri dish containing five black locust bugs was added.

In a second experiment, we tested six wrens held communally in a 1-m^3 cage. A Petri dish containing 15 live black locust bugs was placed on the floor of the cage. Nine minutes later we added a Petri dish containing five crickets and four mealworms. Fourteen minutes later, we added a third dish containing 10 crickets and 10 black locust bugs.

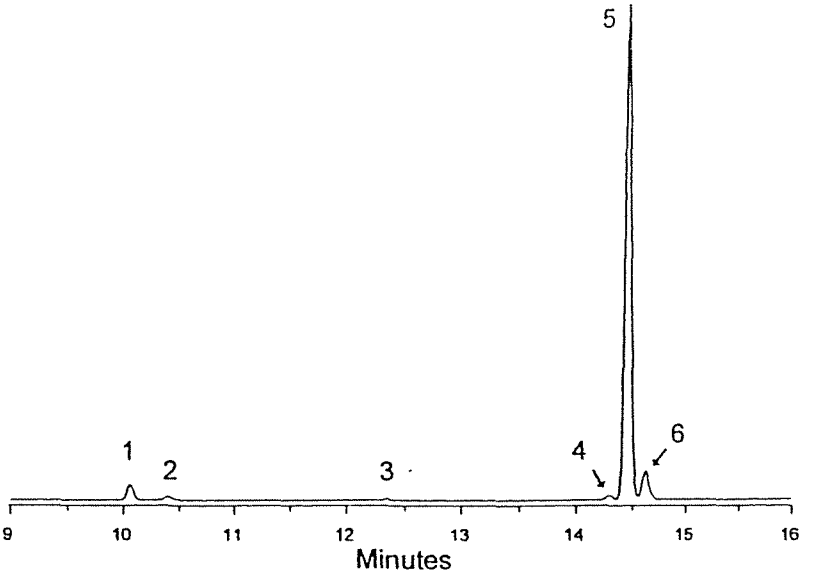
Source of Volatiles

We examined *L. robiniae* adults under a dissecting microscope to determine the presence and location of external gland orifices. We then tested four locations as the possible source of the bugs' odorous secretion: mouth, anus, MTG, and DAG. Two adult males and two adult females were chilled to 10°C. One of each sex was glued ventral side up onto cardstock. The remaining two adults were dewinged using small scissors and glued ventral side down. All bugs were then allowed to warm to room temperature, viewed under a dissecting microscope, and poked or pinched with tweezers to elicit expulsion of the secretion. Slivers of filter paper (ca. 3.0 mm^2) were then placed on the MTG opening, dorsal abdomen, beak, or anus to absorb any ejected fluids, which were then examined for the presence or absence of odor.

RESULTS

Chemical Analysis. No volatiles were obtained from SPME of nonagitated adult male or female *L. robiniae*. Agitated bugs released a blend of at least six different volatiles (Figure 1) that were tentatively identified by matching mass spectra to library spectra. Chemical identification of five of these compounds was confirmed by comparing mass spectra and GC retention times (Table 1, Figure 2) to commercial standards. The sixth compound (peak 4) was identified as (*Z*)-3-octen-1-ol. It matched the authentic standard with respect to both GC retention time and mass spectrum. An authentic standard of (*E*)-3-octen-1-ol was not available for comparison, but by analogy to the hexen-1-ols, the (*E*)- and (*Z*)-3-octen-1-ols should be easily separable by GC, with the *E* isomer eluting first. There was no evidence for the *E* isomer from the bugs.

The relative concentrations of the six identified volatiles were not significantly different between males and females (six separate two-sample *t* tests,



IG. 1. Gas chromatogram of the metathoracic gland volatiles from a male black locust bug, *Lopidea robiniae*.

ich $P > 0.05$). Averaged over both sexes ($N = 12$), the relative mean percentges and standard errors for the six compounds identified were: (*E*)-2-octenal, 5.5% ± 3.3; (*E*)-2-octen-1-ol, 15.5% ± 2.5; (*E*)-2-hexenal, 5.6% ± 1.8; (*E*)-2-exen-1-ol, 2.0% ± 0.8; (*Z*)-3-octenol, 1.2% ± 0.3; (*E*)-2-heptenal, 0.2% ± 0.06.

ABLE 1. COMPOUNDS IDENTIFIED FROM *L. robiniae* (BLACK LOCUST BUG) SECRETIONS AND MS IONS

C peak[a]	Identification	Major MS ions (percentages)
1	(*E*)-2-Hexenal	98 (M^+, 26), 83 (61), 70 (23), 69 (76), 57 (48), 55 (86), 42 (60), 41 (100)
2	(*E*)-2-Hexen-1-ol	100 (M^+, 2), 82 (22), 71 (11), 67 (18), 57 (100), 44 (17), 41 (40)
3	(*E*)-2-Heptenal	112 (M^+, 6), 97 (12), 83 (92), 70 (46), 69 (44), 68 (42), 57 (60), 56 (55), 55 (85), 43, (38), 41 (100)
4	(*Z*)-3-Octen-1-ol	128 (M^+, 1), 110 (14), 95 (15), 81 (63), 68 (53), 67 (43), 55 (100), 41 (51)
5	(*E*)-2-Octenal	126 (M^+, 0.4), 125 (0.9), 108 (4), 97 (14), 83 (61), 70 (90), 69 (46), 57 (58), 55 (100), 42 (42), 41 (100)
6	(*E*)-2-Octen-1-ol	128 (M^+, 1), 110 (8), 95 (12), 85 (8), 82 (24), 81 (29), 68 (31), 67 (26), 57 (100), 55 (47), 43 (41), 41 (56)

[a] Peak numbers correspond to those in Figure 1.

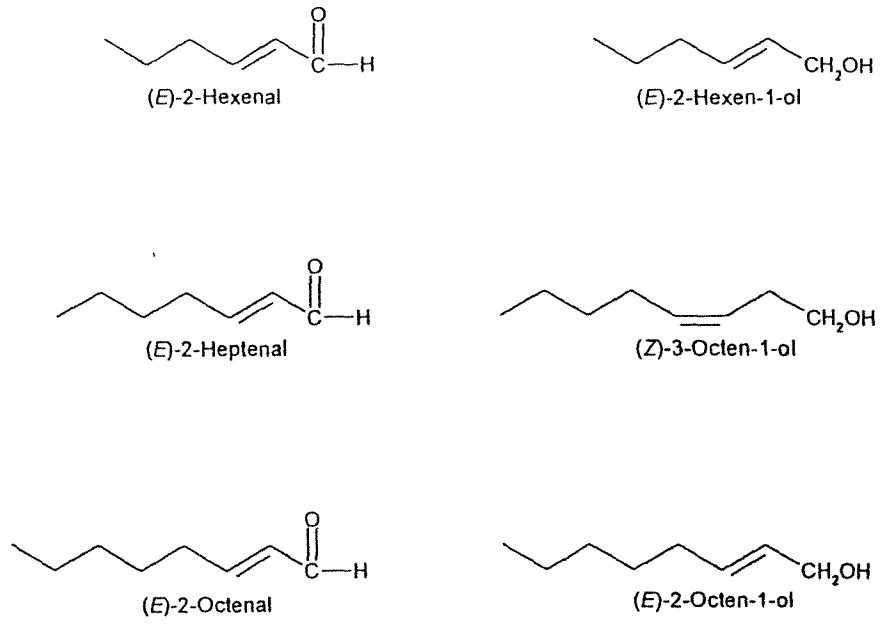


FIG. 2. Compounds identified in *Lopidea robiniae* (black locust bug) male and female metathoracic gland secretions.

Several other minor compounds were noted during GC-MS analysis, but were not identified.

Behavioral Response towards Threats. Nine of 10 adult *L. robiniae* showed defensive behavior when approaching dowels were about 6.0 cm away by quickly crawling away from the dowel to the opposite side of the leaf or stem. Pursuing the bug further with the dowel elicited escape by flight (7 of 10) or dropping (3 of 10), but failed to cause them to expel perceivable volatiles. Four of the 10 bugs secreted when simply caught in the insect net. Five of the remaining six bugs secreted when held between the fingers.

Predator Feeding Trials. All six species of bird tested showed some form of aversion to feeding on black locust bugs, although the exact response varied among individual birds (Table 2). The three birds that had previously encountered black locust bugs (jay, thrasher, robin) consumed only 2 of 15 bugs offered, yet ate 23 of 24 palatable crickets and mealworms. The killdeer and starling, which had not previously experienced *L. robiniae*, initially consumed the bugs, then abruptly stopped and refused all subsequent bugs. The killdeer vomited and the starling gagged (see below). The solitary house wren was also naive yet refused to eat a single black locust bug. Bugs that were attacked by birds emitted their secretion, producing a distinct odor in and near the cage.

TABLE 2. FEEDING RESPONSE OF SIX BIRDS TO BLACK LOCUST BUGS (*Lopidea robiniae*), CRICKETS (*Acheta domesticus*), AND MEALWORMS (*Tenebrio molitor*)

Predator	Bugs (*N*)		Mealworms (*N*)		Crickets (*N*)	
	Offered	Consumed	Offered	Consumed	Offered	Consumed
Blue Jay[a]	5	1	5	5	3	3
Thrasher[a]	5	1	5	5	3	3
American robin	5	0	5	5	3	2
Killdeer[b]	22	12	—	—	10	10
European starling[c]	17	12	8	8	—	—
House wren	16	0	1	1	4	2

[a] Only the first bug encountered was consumed. All subsequent bugs were rejected.
[b] The killdeer ate the first 12 bugs offered, then vomited. Afterwards, it refused all subsequent bugs offered, but ate all crickets offered.
[c] The starling gagged after consuming 12 bugs and 6 mealworms.

The blue jay attacked and swallowed the first bug 46 sec after it was introduced. One minute later we introduced a second bug, which was immediately seized and quickly flung from the beak. At 3 min from the start of the trial, a third bug was offered and was likewise seized and ejected. At 4 min, a fourth bug was introduced; 32 sec later the jay bit and dropped this insect. At 6 min, a fifth bug was introduced. For 5 min, the jay ignored this bug as well as the three previously ejected bugs, which remained in the cage. At 11 min, five mealworms and three crickets were introduced at 1-min intervals. The jay immediately attacked and consumed each, but continued to ignore the bugs remaining in the cage.

The thrasher attacked and swallowed the first bug 3 sec after it was offered. One minute later, a second bug was offered. It was seized within 2 sec and immediately flung from the mouth. Five seconds later the bird picked up the bug again, held it in its mouth for 4 sec and dropped it. At 2 min, a third bug was offered and was immediately seized and dropped. At 3 min, a fourth bug was offered. The thrasher landed next to the bug as if to attack, but turned and flew away. At 4 min, a fifth bug was introduced. The thrasher again landed near the bug, lowered its head as if to attack, then flew away. At 10 min, five mealworms and three crickets were offered at 1-min intervals; all were immediately attacked and consumed. The bird continued to ignore the remaining black locust bugs.

The robin immediately attacked and dropped the first bug offered. During the next 40 sec, it seized and dropped the bug three additional times. At 2 min from the beginning of the trial, a second bug was introduced and was ignored by the robin. At 3 min, a third bug was introduced, and it was immediately seized and dropped. At 4 and 5 min, a fourth and fifth bug were introduced and were ignored. At 10 min, five mealworms and three crickets were introduced at 1-min intervals. The robin quickly consumed the mealworms and two crickets, but continued to ignore the bugs remaining in the cage.

The killdeer ate the first control cricket, demonstrating that it was hungry. The bird was then offered 12 bugs in a row, but was hesitant to eat the first four, which were not eaten until 35–145 sec after they were introduced. However, the next eight were readily attacked and consumed, and each bug expelled its secretion, producing a strong odor. The thirteenth bug offered was rejected, and the second control was then subsequently offered and eaten immediately by the bird. The fourteenth bug was also rejected. The bird then vomited (after consuming 2 crickets and 12 bugs). Thereafter, the bird rejected all subsequent bugs (Table 2), and would (1) bob its head toward its prey as if to attack and then quickly withdraw without touching the prey, (2) actively pace around the bug, or (3) completely ignore the bugs. When a bug flew onto the killdeer's back, it quickly turned its head, bit and released the bug (without harming it), and then began biting the paper on the bottom of the cage. In contrast, the bird immediately consumed all 10 control crickets and, on one occasion, the bird stepped past three moving bugs to get to a cricket.

The starling demonstrated mild aversion towards adult *L. robiniae*. It consumed 12/17 bugs offered vs. 8/8 mealworms (Table 2), but the time to attack was significantly longer for *L. robiniae* (190.9 ± 21.0 sec) than for mealworms (2.3 ± 0.3 sec) (two sample t test, $P < 0.001$; $df = 7$). During the trial, the starling showed aversive behavior toward *L. robiniae*, such as ejecting bugs out of the beak and running toward newly introduced bugs, staring at them from 2 cm away, and then turning away. Near the end of the trial, the bird exhibited a gagging reflex by opening its mouth wide and thrusting its head and neck forward. It then closed its beak and swallowed, which suggests the bird was nauseated and possibly vomited into its esophagus or mouth.

The solitary wren attacked four bugs in quick succession, immediately flinging each bug away. During the next 4 min, it inspected bugs without attacking them. Four minutes into the test, the wren picked up and dropped a bug, then immediately wiped its beak on the floor of the cage. At 10 min, a second Petri dish was added, that contained four small crickets, four black locust bugs, and one mealworm. Within 15 sec, the wren ate two crickets and the mealworm. At 21 min, a third Petri dish was introduced that contained five live black locust bugs. The wren inspected these bugs, but did not attack.

When 15 black locust bugs were placed in a cage containing six house wrens, all birds quickly approached and attacked the bugs. By 5 min after the start of the test, 23 attacks (=bites) had occurred. In 19 of these attacks, the bug was rejected; however, one individual bird consumed four bugs. At 9 min, five small crickets and four mealworms were added to the cage. By 14 min, two mealworms, five crickets, and one of the remaining black locust bugs were eaten. At 23 min, 10 small crickets and 10 black locust bugs were added. By 24 min, nine crickets but no black locust bugs had been eaten. At 27 min, the experiment was concluded with a total consumption of 5 bugs, 2 mealworm, and 14 crickets.

Source of Volatiles. When adult male and female *L. robiniae* were disturbed, an odorous liquid emerged from the MTG openings suggesting that the MTG was the source of the scent. In contrast, filter paper slivers placed on the mouth, anus, or dorsal abdomen of disturbed adults acquired no scent.

DISCUSSION

Chemistry of Lopidea robiniae Secretion. We identified three unsaturated alcohols and three unsaturated aldehydes in the defensive secretion of adult *L. robiniae*. True bugs commonly produce six- and eight-carbon unsaturated (*E*)-alkanes and (*E*)-alcohols (Blum, 1981; Aldrich, 1988; McBrien and Millar, 1999). Hence, the compounds identified in *L. robiniae* represent, for the most part, typical hemipteran exocrine products. Four of the six identified compounds, [(*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (*E*)-2-octenal, and (*E*)-2-octen-1-ol] are common in insect exudates. (*E*)-2-Hexenal is found in the defensive secretions of ants (Crewe et al., 1972), beetles (Tschinkel, 1975), cockroaches (Farine et al., 1997), and in true bugs (Percy et al., 1980; Knight et al., 1984; Hamilton et al., 1985; Staddon et al., 1987; Aldrich, 1988; Borges and Aldrich, 1992; Farine et al., 1992a,b; Leal et al., 1994; Krall et al., 1999; McBrien and Millar, 1999). (*E*)-2-Hexen-1-ol has similarly been identified in the defensive secretions of ants (Crewe et al., 1972), cockroaches (Wallbank and Waterhouse, 1970), and in the pheromone or defensive secretions of numerous Heteroptera (Hamilton et al., 1985; Aldrich, 1988; Smith et al., 1991; Farine et al., 1992a,b; McBrien and Millar, 1999). (*E*)-2-Octenal has been identified in cockroaches (Wallbank and Waterhouse, 1970; Farine et al., 1997), and numerous species of Heteroptera (Percy et al., 1980, Blum, 1981; Knight et al., 1984; Hamilton et al., 1985; Staddon et al., 1987; Aldrich, 1988; Borges and Aldrich, 1992; Farine et al., 1992a,b; Aldrich et al., 1995; Leal et al., 1996; Krall et al., 1997; McBrien and Millar, 1999). (*E*)-2-Octen-1-ol has been found in cockroaches (Wallbank and Waterhouse, 1970; Farine et al., 1997), sawflies (Boeve et al., 1997), and several Heteroptera (Aldrich and Yonke, 1975; Percy et al., 1980; Staddon et al., 1987; Farine et al., 1992a,b; Aldrich et al., 1993; Leal et al., 1996; Krall et al., 1997). (*E*)-2-Heptenal occurs less frequently in insects, relative to the other structurally similar chemicals identified in our research, and has been reported in at least one tenebrionid beetle, *Eleodes beamri* (Tschinkel, 1975) and in the heteropteran families Pentatomidae (Blum et al., 1960) and Cydnidae (Roth, 1961). Trace amounts of both stereoisomers of 3-octen-1-ol (e.g., <1% total secretion) have been found in cockroaches (Farine et al., 1997); however, this compound appears to be exceedingly rare among terrestrial arthropods and, to our knowledge, has never been reported as a primary constituent of any insect defensive secretion.

It is interesting to note that four of the compounds identified in this research are frequently found in a mixture in other Heteroptera. For example,

(*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (*E*)-2-octenal, and (*E*)-2-octen-1-ol are present in the pheromone/defensive secretions of two members of Pyrrhocoridae (Farine et al., 1992a,b). (*E*)-2-Hexenal, (*E*)-2-octenal, and (*E*)-2-hexen-1-ol, are found in the pentatomid *Acrosternum pennsylvanicum* (Aldrich et al., 1995) and are part of a complex defensive secretion in the scutellerid bug *Hotea gambiae* that functioned to deter conspecific competitors and oviposition by parasitoids (Hamilton et al., 1985). Likewise, the former two aldehydes are extremely common in pentatomids (Aldrich et al., 1995; McBrien et al., 2001; Ho and Millar, 2001). The frequent cooccurrence of these compounds suggests a common enzymatic origin, and Blum (1981) has suggested that the alcohols may simply represent precursors to their aldehyde analogues.

Function of L. robiniae Secretion. Our results suggest that *L. robiniae* is chemically defended and that the volatiles found in the MTG secretion of *L. robiniae* function in antipredator defense. We base these conclusions on the following: (1) The secretion is discharged in direct response to threats. (2) Both sexes produce the same major components in the secretion, suggesting that they do not function as sexual pheromones. (3) Adults are brightly colored, implying an aposematic warning function (Pasteels et al., 1983; Guilford, 1990). (4) Adult black locust bugs occasionally aggregate, a trait often associated with chemically defended insects (Pasteels et al., 1983). (5) The types of compounds identified in the MTG secretion (short-chain aldehydes and alcohols) are volatile, highly odorous, and are known to have toxic or irritating effects (Eisner, 1970; Hamilton et al., 1985; Griffin and Segall, 1989; van Iersal et al., 1997). Hence, they are ideally suited to serve as alerting or punishing substances (Blum, 1981; Whitman et al., 1990) and can effectively deter predators (Blum, 1981; Hamilton et al., 1985; Aldrich, 1988; Gunawardena and Herath, 1991; Gunawardena and Bandumathie 1993; Krall et al., 1999). Finally, (6) Several predators tested in this study demonstrated feeding aversions towards *L. robiniae*. For example, after sampling a single bug, the jay and the thrasher rejected all others. The robin and several wrens refused to eat a single bug. Two birds exhibited symptoms of toxicosis after consuming black locust bugs: the killdeer vomited and the starling gagged. The most telling response was seen in the robin and the solitary wren: both birds eagerly attacked the first bugs offered, but then immediately ejected the bugs from their beaks, suggesting that the secretion itself was unpleasant. After biting bugs, the solitary wren wiped its beak on the floor of the cage, and the killdeer bit paper. These behaviors suggest that the secretion was irritating or unpleasant and that the birds were attempting to remove the offending secretion from their mouths.

It is interesting that the bird predators we tested displayed inter- and intraspecific variation in response to black locust bugs; some birds refused to attack, some ate only one bug, and some consumed several bugs. Some bugs were killed during the attacks, whereas other bugs survived relatively unscathed. Such differences in predator response to a single prey species are frequently observed in predator–prey

interactions (Brower, 1984; Whitman et al., 1985; Krall et al., 1999). Variability in predator response and prey survival is important for the evolution and continued maintenance of antipredator defenses in prey. It suggests that even limited or low-impact defenses would have selective advantages against timid predators. It also suggests that even a well-defended prey species would continue to experience selective pressure from naive, more aggressive, or less sensitive predators.

Chemicals in defensive secretions can function in different ways (Whitman et al., 1990), and it remains to be determined exactly how the compounds identified in this research facilitate prey rejection by bird predators. Defensive chemicals can be noxious Class I compounds, which poison, harm, or irritate predators, or relatively innocuous Class II compounds, which typically are not toxic themselves but function as chemical warning signals for other toxic compounds (Brower, 1984). Our observations suggest that secretions from *L. robiniae* may work on both levels. For example, the secretion appeared to be an immediate repellent to the robin and the solitary wren, which repeatedly spit out bugs without consuming them. Alternatively, the killdeer seemed little affected by the "taste" of the bug and appeared to consume them (at first) with impunity. However, this predator later vomited, suggesting that the black locust bug may possess internal toxins in addition to a secretory defense. Indeed, many chemically defended insects, exclusively or in addition to the use of volatile secretions, employ slower-acting, high-molecular-weight internal toxins, which are often sequestered directly from toxic host plants (Duffey, 1977; Blum, 1981; Aldrich, 1988; Bowers and Farley, 1990; Aldrich et al., 1997; Aliabadi and Whitman, 2001). Black locust trees contain a number of potent toxins, including the phytotoxin robin, the glycoside robitin, the alkaloid robinine, and numerous phenolics (Lewis and Elvin-Lewis, 1977; Duke, 1985; Bruneton, 1999). The consumption of black locust bark, leaves, or seeds causes serious neurointenstinal reactions in humans (especially children) and livestock (Kingsbury, 1964; Tampion, 1977; Stevens, 1980; Duke, 1985; Harborne and Baxter, 1993). Several Heteroptera appear to sequester defensive compounds from host material (Aldrich, 1988; Aliabadi and Whitman, 2001), presumably for chemical defense. Possibly, black locust bugs, which are thought to feed almost exclusively on black locust, may sequester plant toxins for their defense. Hence, *L. robiniae*, like many other chemically defended insects, might posses a paired defense mechanisms: an initial defense that warns or deters, backed up by an internal defense that is the primary basis for their unpalatability.

Sequestration of host plant toxins might explain the apparent mimicry relationship between the black locust bug and the locust leaf miner. At present it is not known which species is the model or the mimic, if the relationship is Batesian or Mullerian, or even if a true mimicry relationship exists. However, it is possible that both species sequester defensive substances from their common host plant.

In addition to a primary defensive role, the MTG secretions of some true bugs also function as sexual, aggregation, alarm, or dispersal pheromones (Aldrich,

1994, 1996; McBrien and Millar, 1999). This is true in the Miridae, where sexual pheromones from the MTG gland are known for a number of species (Knight et al., 1984; Thislewood et al., 1989; Aldrich, 1988, 1996; Millar et al., 1997; McBrien and Millar, 1999). Although *L. robiniae* occasionally aggregate, it is not yet known if their MTG secretions exhibit pheromonal activity.

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